FUNGI ASSOCIATED WITH *BORRICHIA FRUTESCENS* (ASTERACEAE): INSECT GALLS AND ENDOPHYTES

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ABSTRACT

This report compares fungi found in galled and non-galled plants of *Borrichia frutescens* (L.) DC., Asteraceae. Fungi were observed in the plant galls of the midge, *Asphondylia borrichiae* Rossi & Strong. A variety of endophytic fungi were cultured from the apical meristems, stems and leaves of galled and non-galled plants collected from several coastal sites in Florida. Fifteen percent of midges examined ($n = 60$) carried fungal spores of *Alternaria* sp.

RESUMEN

Este artículo compara los hongos encontrados en plantas con y sin agallas de *Borrichia frutescens* (L.) DC., Asteraceae. Se observaron hongos en las agallas de *Asphondylia borrichiae* Rossi & Strong. Se cultivó una variedad de hogo endofítico de los meristemos apicales, tallos y hojas de plantas con y sin agallas colectadas en varios lugares costeros de Florida. El quince por ciento de las agallas examinadas ($n = 60$) tenían esporas de *Alternaria* sp.

INTRODUCTION

Borrichia frutescens (L.) DC. or bushy seaside oxeye is a perennial herbaceous Asteraceae often found bordering the salt marsh communities of the Gulf of Mexico and Atlantic Ocean. Insect galls on this host species are initiated in the apical meristem by a midge, *Asphondylia borrichiae* Rossi & Strong (1990). These galls are also impacted by four specific parasitoid wasps (*Galeopsomyia haemon* Walker, *Rileya cecidomyiae* Ashmead, *Tenuipetiolus teredon* Walker, and *Torymus umbilicatus* Gahan). Individuals from these parasites lay their eggs inside the gall with *A. borrichiae*. The parasitoids devour the developing midges and significantly affect population levels of *A. borrichiae* (Rossi et al. 1992). Several biological studies on this plant midge system elaborate on the life history and ecological details including parasitism rates (Rossi & Strong 1990; Stiling et al. 1992; Stiling 1994; Rossi & Stiling 1998; Rossi et al. 1999). As in many insect galls, the tissue surrounding the developing larvae eventually becomes full of chambers layered with fungal growth purportedly providing nutrition for the developing larvae (Gagne 1989). Thus, knowledge of the fungi associated with both the galled and non-galled plants is needed to gain insight into this host-parasite relationship.

It is unclear how fungi are introduced into the galls in the *B. frutescens* system. Many plants are known to harbor microbial endophytes which predomi-

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nantly are fungi. These organisms live internally in plant tissue. Stone et al. (2000) suggest that these endophytes secondarily invade insect galls. Batra and Lichwardt (1963) indicate that "gall fungi are airborne, grown in a variety of substrata and are not species specific." Other investigators of insect galls suspect that spores of fungi are accidentally collected from leaves and leaf litter by the insects (Borkent & Bissett 1965). Haridass (1987) suggests that fecal contamination is another possible means of spore dispersal whereby fungi are introduced into the plant tissue. Gagne (1989) reports that midges feed by sucking on the hyphae and states, "fungi are evidently obligatory in all galls...and are food of those gall midges." Batra and Lichwardt (1963) alternatively indicate that it is difficult to determine whether midges actually feed on the hyphae or the plant tissue degraded by the fungi.

In this study we examined a number of midge galls of *Asphondylia borrichiae* from *Borrichia frutescens* from several Florida sites for the presence or absence of fungi. In addition plant apices, stems, and leaf samples of galled and non-galled plants were cultured for fungi. Finally, a small population of midges was surveyed for associated fungal spores.

MATERIALS AND METHODS

Sites for Collections.—*Borrichia frutescens* galls, leaf and stem samples were collected from the following locations from 1993–1995 in Florida:

Site #1–Merritt Island National Wildlife Refuge, Titusville (Brevard County).

Site #2–Little Jetties Park, Mayport (Duval County).

Site #3–Ft. DeSoto State Park, St. Petersburg (Pinellas County)

Site #4–Upper Tampa Bay Park (Hillsborough County).

Site #5–Delnor-Wiggins State Park, Naples (Collier County).

Culture Protocol.—In May, 1995, five samples each of apex, stem, and leaf tissue were collected from galled and non-galled plants from Sites 1, 2, and 3. All collected plant samples were placed in individual, sterile plastic bags, refrigerated, and returned to the laboratory within three days. Excised tissue from apices, stem and leaf tissue of both galled and non-galled plants tissues were rinsed in running water for 5 min, then each tissue type was placed in separate flasks and rinsed with 95% ethanol for 2 min. This was followed by a 30 min shake rinse in 20% bleach solution. All tissues were held in flasks of sterile distilled water (1- 2h) while tissues were prepared for incubation. Three slices from each tissue from each site were submerged in Potato Dextrose Agar (PDA) and V-8 Juice Agar (Difco, Detroit, MI). These cultures were placed in a dark incubator at 32°C for seven days. Isolates from successful cultures were placed on PDA slants and held at ambient temperature until sporulation after which identifications were made.

In Situ Fungal Studies.—In September, 1995, ten stems were collected randomly from galled and non-galled plants of *B. frutescens* from four sites (# 1-4). Each stem was cut into 1.5 cm segments. Using the protocol established by Hignight et al. (1993) stems were cleared and then all segments were microscopically examined for the presence of endophytes.

Direct Observations of Insects.—Whole insects of *Asphondylia borrichiae* were collected as they emerged from galls of *Borrichia frutescens*, ($n = 60$), *Iva imbricata* Walter ($n = 20$), and *Iva frutescens* L. ($n = 5$) and preserved in vials of 95% ethanol. All insect samples were examined for the presence or absence of fungal spores on the surface using light microscopy.

RESULTS AND DISCUSSION

Previous dissection of the *Borrichia frutescens* galls revealed larval chambers (pers. obs.). Within these white mycelium could be observed and this fungal growth was arrested until the larvae became fully developed. Then the mycelial mat became denser, darker, and quite distinct (Fig. 1).

Midge galls observed in the Asteraceae, and fungi isolated from those galls are listed in Table 1. In addition, Farr et al. (1989) note the occurrence of *Aecidium borrichiae*, *Puccinia mirifica*, and *Cercosporidium* sp. on stems and leaves of *B. frutescens*. Although many plants have fungal endophytes (Clay et al. 1985; Clay 1990; Carroll 1988), prior to this they were not known from *B. frutescens*.

Preliminary studies (1993–1994) including direct observations and excising and culturing of a range of ages of gall tissue from *B. frutescens* from all five sites consistently yielded a large variety of fungi including two types of sterile mycelium (data not shown). Similar observations have been made in other studies of midge galls. Bissett and Borkent (1988) report that some midges from the Lasiopterini and Asphondylidi inoculate galls with a variety of mitosporic fungi and use them as a food source. Stone et al. (2000) observed that galls on Douglas-fir (*Pseudotsuga menziesii*) support heavy fungal growth which, he suggests, may be plant pathogens in addition to providing insect nutrition. Wilson (1995) proposes that fungi in galls may be saprobes or inquilines (organisms inhabiting insect galls not parasitizing the gall maker but otherwise utilizing the gall tissue for food).

To determine if endophytic fungi could be observed microscopically and using the method of Hignight et al. (1993), a preliminary study was conducted in May 1995 using five stems each from galled, non-galled, flowering non-galled and flowering galled plants of *B. frutescens* collected from Site #3, Ft. Desoto. This mini-study revealed that endophytic fungi were present in 100% of the samples from plants with galls appearing similar to the example seen in Figure 2. The remaining non-galled stems were less heavily colonized with fungi. This qualitative observation led to additional studies to quantify endophytic fungi

FIG. 1. *Borrichia frutescens*. Cross section through a gall showing the layer of the fungal mycelium.

involved in the stems of galled and non-galled plants. We observed a consistent occurrence of endophytic fungi in all sampled stem segments from four diverse Florida locations (Sites 1–4). In all non-galled plants these occurrences range from 20 to 64% and in galled plants 30 to 64%. This suggests little influence of the role of the midge. But one might argue that the fungal endophytes in galls grow out, invading other plant tissue. On the other hand, depending on the time-frame, endophytic fungi may grow into the gall or exist in the pre-gall tissue and contribute to the mycoflora of the aging gall. Either way, fungal endophytes are extant in *B. frutescens*.

In the apices, stems, and leaves of both galled and non-galled plants from Sites 1, 2, and 3 several fungal taxa occur (Table 2). Included among the isolates are several types of sterile mycelium. These were found among all tissue types. *Alternaria* sp. was also found among all tissue types (Table 2). *Acremonium strictum*,

TABLE 1. Fungi reported in gall midge associations in Asteraceae.

Gall midge	Host	Fungi	Reference
Asteromyia carbonifera	*Solidago canadensis*	*Sclerotium asteris*	Weis 1982; Batra 1964
A. tumifica, A. modesta *A. carbonifera*	*S. mollis*	*Macrophoma* sp.	Borkent & Bissett 1985
A.carbonifera	*Aster* sp.	*Rhytisma asteris*	Batra 1964
A.carbonifera	*S. sempervirens*	*R. bifrons*	Batra 1964
A.carbonifera	*S. lanceolata*	*R. solidaginis*	Batra 1964
A. ratibidae	*Ratibida columnifera*	*Chaetomium globosum*	Batra 1963
A. ratibidae	*R. columnifera*	*Aureobasidium pullulans*	Batra 1963
A. ratibidae	*R. columnifera*	*Plectosporium tabacinum* (as *Cephalosporium ciferrii*)	Batra 1963
A. carbonifera	*S. graminifolia*	*Alternaria* sp.	Batra 1963
Bucculatrix simulans	*Helianthus* sp.	*Trichoderma viride*	Batra 1963
B. simulans	*Helianthus* sp.	*Aspergillus* sp.	Batra 1963
B. simulans	*Helianthus* sp.	*Trichothecium roseum*	Batra 1963
B. simulans	*Helianthus* sp.	*Penicillium* spp.	Batra 1963
B. simulans	*Helianthus* sp.	*Stemphylium* sp.	Batra, 1963
B. simulans	*Helianthus* sp.	*Botrytis cinerea*	Batra 1963

Bipolaris sp., and *Verticillium lecanii* displayed more irregular distribution in the plant tissues from the sites sampled. Other species of these genera have been reported in the endophytic fungal literature (Bacon & White 2000).

The mycangia (small special pockets adjoining the abdomen) of midges in the wild are known to be filled with fungal spores that are thought to be inserted with the eggs at oviposition. In the newly emerged midges examined in this study, no spores were seen in the mycangia. Spores of *Alternaria* sp. were seen on the surfaces of 15% of the midges emerging from *Borrichia frutescens* galls (n = 60); 10% of the midges from *Iva imbricata* (n=20); and 40% of these midges from *I. frutescens* (n = 5). Limited observations of several epidermal peels from plants of *B. frutescens* revealed that *Alternaria* sp. and *Fusarium* sp. were abundant on the plant surface. While it may be possible for the midges to carry fungal spores at oviposition, it seems that the diversity of fungi seen in these midge galls and host plant tissue are sourced by other means, possibly including some of their parasitoids.

We observed that fungal endophytes appear to become denser in the plant tissues between May (seen in the preliminary study) and September revealing increasing hyphae and pigmentation (Fig. 2). The aging galls in this study were seen to include fungal contents that became denser and darker with pigments (Fig. 1). *Alternaria* sp. and *Bipolaris* sp., both darkly pigmented, were consistently

TABLE 2. Percent occurrence of fungi isolated from apical, stem and leaf tissues isolated from *Borrichia frutescens*: galled and non-galled plants.

	Merritt Island		Mayport		Ft Desoto	
	gall	non-gall	gall	non-gall	gall	non-gall
Sterile mycelia						
Apex	33.3	–	66.7	13.3	40	–
Stem	33.3	20	26.7	20	–	–
Leaf	6.7	26.7	13.3	6.7	40	–
Alternaria sp.						
Apex	–	13.3	–	–	13.3	–
Stem	6.7	–	–	–	–	–
Leaf	20	6.7	33.3	6.7	–	13.3
Verticillium lecanii (A. Zimmerm.) Viégas						
Apex	–	–	–	–	–	–
Stem	–	–	6.7	–	–	–
Leaf	–	20	–	–	20	–
Bipolaris sp.						
Apex	–	–	–	–	–	–
Stem	6.7	–	–	–	–	–
Leaf	–	6.7	–	–	–	–
Acremonium strictum W. Gams						
Apex	–	–	–	–	–	–
Stem	13.3	–	20	–	–	–
Leaf	–	–	–	–	–	–
Humicola grisea Traaen						
Apex	–	–	–	–	–	–
Stem	–	–	–	–	6.7	–
Leaf	–	–	–	–	–	–
Monocillium indicum S.B. Saksena						
Apex	–	–	–	–	–	–
Stem	–	–	–	–	6.7	–
Leaf	–	–	–	–	–	–
Penicillium sp.						
Apex	–	–	–	6.7	–	–
Stem	–	–	–	–	–	–
Leaf	–	–	–	–	–	–
Fusarium sp.						
Apex	–	–	–	–	–	–
Stem	6.7	–	–	–	–	–
Leaf	–	–	–	–	–	–

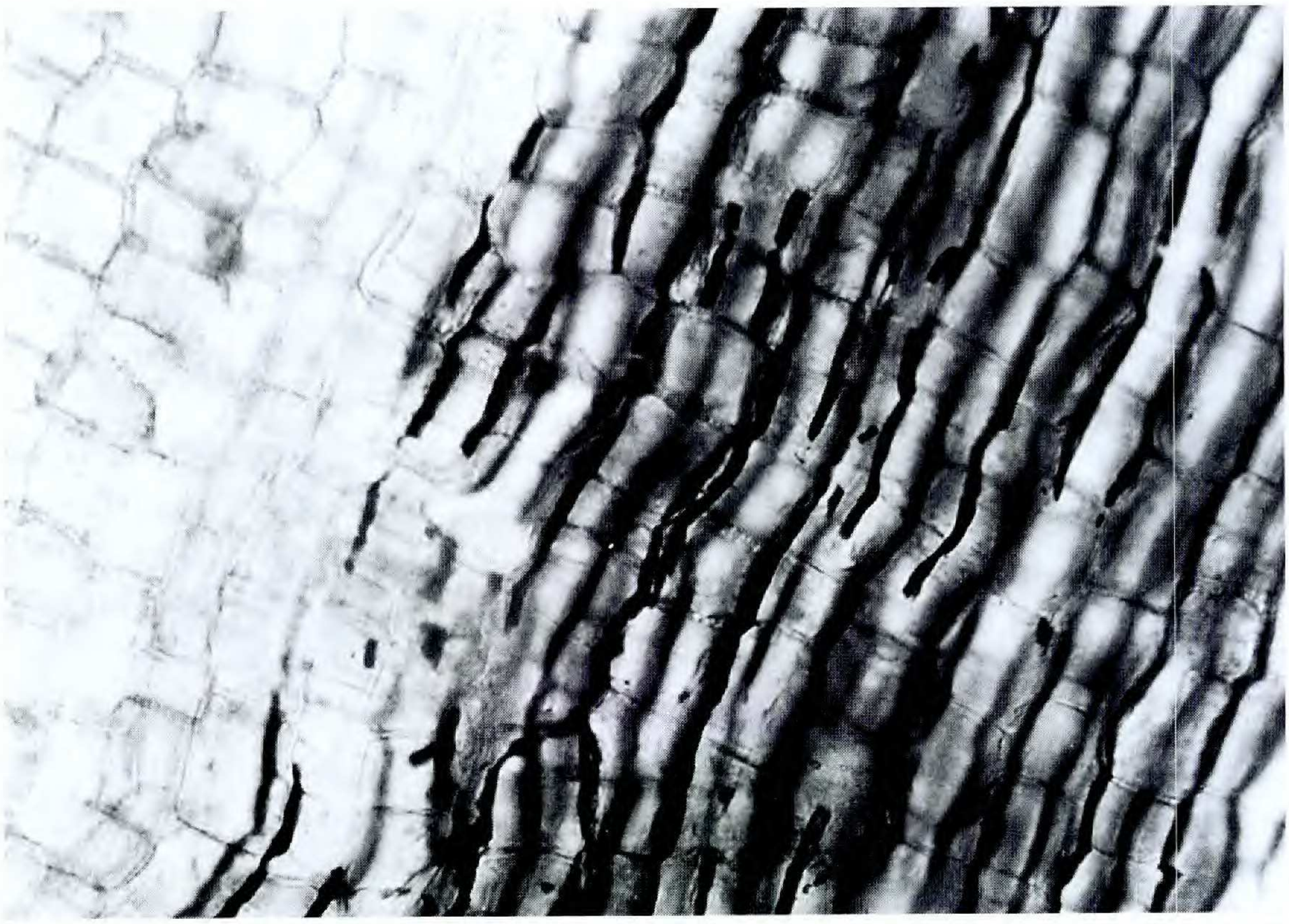

FIG. 2. *Borrichia frutescens.* Hyphae of endophytes in stem pith tissue.

isolated from galled and non-galled plant tissue. Shaw (1992) reviewed several studies indicating that fungivores consistently favored darkly pigmented litter fungi. Interestingly, it seems that *Alternaria* sp. and *Bipolaris* sp. could play a nutritional role in this gall system.

In this study we establish the presence of entophytic fungi in insect galls. Endophytic fungi were cultured from tissues of galled and non-galled plants of *Borrichia frutescens*. Both of these plant types carry several fungal taxa. These observations are common to all sites, but sample sizes are too limited to discern major differences. Fungal spores were directly observed on 15% of examined midges. This suggests that the midges may play a role in fungal dispersal to gall tissue as proposed by Batra and Lichtwardt (1963).

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